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OFF-THE-SHELF ANALYZERS FOR MEASURING ADENOSINE TRIPHOSPHATE (ATP) IN ACTIVATED SLUDGE

NATIONAL ENVIRONMENTAL RESEARCH CENTER

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"OFF-THE-SHELF" ANALYZERS FOR MEASURING ADENOSINE TRIPHOSPHATE (ATP) IN ACTIVATED SLUDGE

by

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Program Element 1BB043

NATIONAL ENVIRONMENTAL RESEARCH CENTER
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FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment--air, water, and land. The National Environmental Research Centers provide this multidisciplinary focus through programs engaged in

- studies on the effects of environmental contaminants on man and the biosphere, and
- a search for ways to prevent contamination and to recycle valuable resources.

This study describes and compares commercially available analytical instrumentation of potential value in improving the control of those wastewater treatment plants which employ the activated sludge process. Currently, this process is the Nation's most widely used method for biological secondary treatment of domestic wastewaters. Consequently, any significant improvement in control of activated sludge treatment could result in more efficient plant operation with attendant cost savings and/or improvements in effluent quality.

A. W. Breidenbach, Ph.D.
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National Environmental
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ABSTRACT

A desktop survey was made of commercially available and relatively inexpensive adenosine triphosphate (ATP) analyzers; one of these appears particularly suitable for monitoring percent viable biomass (or cell activity) in biological waste-treatment processes. Costs of required equipment and reagents are presented, and an extensive bibliography of various applications of ATP analyses is also furnished.

SUMMARY AND CONCLUSIONS

For STP monitoring and control applications, currently only two off-the-shelf ATP analyzers are both available and practical; these are duPont's Biometer and JRB Incorporated's ATP Photometer. Both of these instruments are manually operated devices capable of furnishing a new ATP measurement every 10 minutes, with lighted digital display of the electronically processed analytical data. The ATP Photometer requires the use of a calibration curve; the Biometer does not. On the other hand, reagent costs for the Biometer are about 10 times those for the ATP Photometer, and the initial capital outlay for the Biometer is about $4,860 compared with $3978 for the ATP Photometer (really, about $4200 instead of $3978 if adjustment is made for the Biometer's larger initial supply of reagents, cuvettes, pipetor tips, and furnished auxiliary hardware). The ATP Photometer's luminescence-integrating principle should make it less susceptible than the Biometer to minor operational variations from sample to sample, and also less susceptible to errors stemming from slight variations in batch-to-batch reagent purity.

Thus, for most STP applications, both JRB's ATP Photometer and Dupont's Biometer appear to be applicable with JRB's instrument possessing certain advantages.

INTRODUCTION

For several years, investigators have been pointing out the inadequacies of "classical" biological monitoring and control tests used in biological waste treatment (1, 2, 3). Patterson et al. (1) documented a particularly strong case against basing control of the activated sludge process on tests such as BOD (or COD, TOC, and TOD), MLVSS, sludge age, sludge yield, and biochemical oxidation rate constants. Instead, these authors recommend use of direct measurements of overall cell activity or of a closely related parameter which is now referred to as viable biomass, both of which are more reliable indicators of a sludge sample's assimilative capability for non-toxic organic wastes than are any of the classical measurements.

Cell activity or viable biomass can be related to measurements of either oxygen-uptake rates or certain cellular enzymes such as adenosine triphosphate (ATP) or the two dehydrogenases, flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD). Although measurements of FAD and NAD are relatively simple to perform, theoretically neither measurement can be expected to yield as reliable an indication of cell activity as is theoretically possible through careful measurements of either ATP or oxygen-uptake rate. Colorimetric determination of FAD by reaction with triphenyl tetrazolium chloride (TTC) not only yields an insoluble precipitate at the reaction site, which may thus interfere with normal oxygen-uptake reactions, but this particular assay for cell activity automatically bypasses two-thirds of the oxidative phosphorylation reactions in the electron-transfer chain governing cell respiration. Both of these factors are potential sources of error in determining true cell activity with TTC. On the other hand, colorimetric determinations of NAD by reaction with phenazine methosulfate bypass all the sites of oxidative phosphorylation in the electron-transfer chain and again, introduce serious potential sources of error in measuring true cell activity by that approach.

Direct measurement of oxygen-uptake rate has none of the above disadvantages since it is a measurement of the final electron acceptor (i.e., oxygen) in the respiratory electron-transfer chain. Until quite recently, however, reliable oxygen-uptake devices suitable for automatic on-line process monitoring and control were not commercially available. Currently, at least four on-line devices have been invented for this purpose: Badger Meter Company's Biological Respirometer, Union Carbide Corporation's Biomonitor, Robertshaw Controls Company's Continuous Bacterial Respirometer, and a custom-made respirometer

now being used by Dow Chemical Company at an EPA grant location at Freeport, Texas. One of Badger's Biological Respirometers has been purchased for field evaluation by an EPA grantee at Palo Alto, California. The Robertshaw device is also commercially available, but it is a relatively new instrument for which limited field-evaluation data exist. Carbide's Biomonitor has not yet been licensed to a commercial instrument firm for manufacture and sale to the public; however, Carbide claims that the Biomonitor soon will be a commercially available instrument. Future commercial plans for the Dow device have not been announced.

Determination of cell activity through measurements of cellular ATP is now widely practiced, particularly in the fields of biology, oceanography, pharmacology, and clinical chemistry (see attached bibliography). This same concept has also been gaining acceptance, since about 1968, as a promising tool for improving the performance of biological waste-treatment processes, especially those employing activated sludge. ATP seems to be a unique biological "indicator" for several reasons:

1. It is found in all living material, yet it rapidly disappears when an organism dies.

2. For any given type of microorganism, the ATP content per cell is relatively constant over most of the cell's growth phase. Kao et al. (4) recently described this characteristic in detail for pure and mixed cultures of E. coli and P. aeruginosa.

3. A study of 13 common bacterial species showed that the mass of ATP/mass of living cell material is approximately constant; it averages about 0.5×10^{-15}g of ATP/cell.

Thus, a rapid live-cell count can be determined using the average value of 0.5×10^{-15}g ATP/cell, plus the measured ATP concentration of the biological system in question. An analogous approach permits calculation of a sample's percent viable biomass, again using measured values of the sample's ATP concentration.

In 1969 and 1970, Patterson et al. (loc. cit.) proved the specificity and reliability of activated sludge ATP measurement as an indicator of cell metabolic activity, viable biomass, and shock loadings of such toxicants as heavy metals and strong acids. In 1971, Brezonik and Patterson (5) presented additional correlations of activated sludge ATP concentration with sudden stresses on such systems caused not only by acids and heavy metals, but also by large

temperature increases, drastic reductions in dissolved oxygen (DO) concentration (including brief periods of sludge anoxia), and broad variations in available substrate.

The article by Brezonik and Patterson further substantiated previous reports (2, 6) that only a relatively small fraction (about 15 to 20%) of activated sludge mass ordinarily is viable in a full-scale activated sludge unit; whereas, on a carefully controlled pilot scale, this fraction may approach 45 to 50%. These findings could be very important to sewage treatment plant operators who are attempting to achieve optimum aerator performance by control of F/M (i.e., food/microorganism) ratio through adjustments in sludge return rate. For such locations, reliable estimates of percent viable biomass in sludge being returned to the aerator should greatly enhance the accuracy of F/M control settings, as well as the utility of the resulting process data. Routine determinations of return sludge activity by means of rapid on-site ATP measurements (i.e., 10 minutes/ determination) would seem to offer a practical and reliable route to accurate F/M control.

At the present time, all off-the-shelf ATP analyzers (described in detail in the next section) are manually operated devices. Nevertheless, at least one, large, metropolitan sanitary district (City of Denver, Colorado) is studying the potential of ATP measurement for monitoring and controlling the activated sludge process. This municipality is interested in employing the manually operated, JRB Incorporated, ATP Photometer for their studies because of this instrument's low operating costs and rapid, automatic, digital readout.

There is no foreseeable technical obstacle to automating at least three existing ATP devices; in fact, Travenol Laboratories (a pharmaceutical supply house at Morton Grove, Illinois) has already automated American Instrument Company's Chem-Glo Photometer for their inhouse R&D needs. Both JRB and duPont claim that automating their ATP instruments should be a relatively easy job if sufficient funds were available for modest amounts of developmental work. Although duPont has stated that they have no plans to carry out such developmental work at their own expense, they have given Union Carbide Corporation a Biometer in exchange for one of Carbide's Biomonitors, and there is every indication that Carbide may automate duPont's Biometer for their own needs should the instrument's performance warrant the required R&D effort.

Analyses for ATP can be carried out in at least two different ways:

I) ATP + glucose (excess) + hexokinase ——> glucose-6-phosphate + ADP,

glucose-6-phosphate + glucose-6-phosphate dehydrogenase + $NADP^+$ ⟶ 6-phosphogluconic acid + NADPH + H^+

Leese and Bronk (7) showed how the extent of fluorescence of NADPH formed in this reaction gives a measure of the amount of ATP originally in the sample. These investigators used modified AutoAnalyzer equipment for their work.

II) luciferin + ATP + luciferase $\xrightarrow{mg^{++}}$ pyrophosphate + luciferyl-adenylate (a luciferase-luciferin-adenosine monophosphate complex),

luciferyl-adenylate + O_2 ⟶ "Product" + luciferase-luciferin-O^*,

luciferase-luciferin-O^* ⟶ luciferase-luciferin-O^- + light ($h\nu$).

The initial, very brilliant, burst of light ($h\nu$) rapidly declines in about 3 seconds to a low, but uniform, level of luminescence, which persists for well over 3 minutes. Suitable measurements, either of the initial light burst or of the residual low-level luminescence, gives a measure of the ATP originally in the sample.

All of the presently available, off-the-shelf, ATP analyzers employ reaction sequence number II (known colloquially as the "firefly reaction"), probably because of its extremely high sensitivity and its relatively low reagent-cost per analysis. For example, cost per analysis usually ranges from about 5¢ to 40¢, depending upon the purity of firefly-lantern extract required by the analyzer being used. However, whether reagent cost is 5¢ or 40¢, the usual limit of detection is about 10^{-10}mg ATP, and the practical limits of analysis are about 10^{-9}mg ATP/ml up to 1 mg ATP/ml. At ATP concentrations approaching the upper practical limit of analysis, sample dilutions may be required (for those cases where it is not feasible simply to use smaller sample sizes) to avoid "swamping" the analyzer's photodetection system with too much emitted radiation. On the other hand, if the analyzer is equipped with automatic range-changing circuitry (e.g., duPont's Biometer), the necessity for sample dilution is minimized.

Regardless of which manufacturer's analyzer is involved, determination of cell activity or viable biomass consists of a five-step procedure: reagent preparation, instrument

calibration, extraction of ATP from the biomass, measurement of the ATP, and data processing. Some instruments automatically process the analytical data on a real-time basis and either display or print out the parameter of interest; this obviously saves the operator considerable time and permits faster repetitive analyses. However, even the first four steps, above, are not as foolproof as they may appear. For example, some of the reagents must be prepared and subsequently handled in carefully prescribed ways if instrument calibration and sample analysis are to be meaningful. Instrument calibration per se can be straightforward and reliable, but again this is the case only if the luciferin-luciferase reagent has been suitably aged in an ice-bath (to permit die-away of unwanted background radiation) and if the highly unstable standard solutions of ATP are either used immediately after preparation or stored at -20°C until needed. Even the extent of extraction of cellular ATP from the sample being assayed is sensitive to procedural variations, although some extraction methods are inherently less susceptible to such errors than are alternative methods. Reproducible measurement of sample ATP requires close attention to techniques with those instruments, such as duPont's Biometer, which measure only peak intensity (i.e., the initial, brilliant, 2-or 3-second flash), rather than post-flash, long-term, dieaway luminescence. "Peak-intensity instruments" require careful sample-cell alignment for each new measurement, not only to ensure optical-path reproducibility but also to ensure reproducibility of mixing at the moment each sample is injected (thru a "blind sampling" port) into the prepositioned cell within the analyzer. "Luminescence-integrating instruments," such as JRB's ATP Photometer that measures long-term dieaway luminescence, are likewise susceptible to optical cell misalignment; however, since sample mixing takes place before the optical cell is placed out of sight within the analyzer, errors due to slight variations in sample-injection and sample-mixing techniques are virtually eliminated.

In 1970, NASA's Langley Research Center published a report (8) describing a bacterial detection system that appeared to circumvent many of the above difficulties involved with bioluminescent ATP measurements of live cell concentration. This NASA analyzer employed chemiluminescent measurements of cellular cytochrome-c concentration by reacting this latter with alkaline luminol in the presence of sodium perborate. Unfortunately (and as is typically the case for most such chemiluminescent assay methods), the NASA procedure permitted reliable estimates of bacterial concentration only for biological specimens containing a single bacterial species. Furthermore, information correlating light emission to bacterial concentration had to be available, even for that particular species, if quantitative estimates were

required. NASA has since reverted to the use of the bioluminescent firefly reaction for their more recent development of live-cell-count analyzers, such as FLASH (i.e., Fast luciferase Automated Assay of Specimens for Hospitals). The FLASH device does not yet appear to be available as an off-the-shelf item.

PRESENTLY AVAILABLE EQUIPMENT

DuPont's "760" Luminescence Biometer - This is a small, highly sensitive, single-beam photometer (15 x 16 x 7.5 in.; 38 x 41 x 19 cm.) weighing only 23 lb (10.4 kg). It possesses a fixed wavelength range, extending from 350 nm (near ultraviolet) to 600 nm (visible region), specifically selected for measurement of radiation from bioluminescent or chemiluminescent reactions. With the use of specially made (or purified) duPont reagents, the instrument can detect 10^{-13}g ATP/0.01 ml of injected sample. The instrument also has automatic ranging, five-decade, linear response capability that allows most samples to be measured directly without a predilution step. Contrary to the competing ATP analyzer marketed by JRB Incorporated, the Biometer measures only the peak intensity of a luminescent reaction; i.e., it has no integrating circuitry for measuring the total amount of radiation emitted over a sustained time interval. The Biometer's analytical readout includes a lighted digital display of either ATP concentration or cell concentration, the choice of which is governed by a selector switch. Also furnished is a 100-mv recorder-output signal. Instrument-power requirement is only 0.2 amp. at 105-130 V and 50-60 Hz; no other supporting utilities are needed.

Reagent preparation and Biometer calibration are simplified by the use of special reagent kits supplied by duPont. For example, to prepare a reaction mixture, a tablet containing buffer and magnesium sulfate is dissolved in water. A premeasured, homogeneous, powdered mixture of luciferin and luciferase is then added to the buffered magnesium sulfate solution, and this reaction mixture is dispensed into cuvettes. Each cuvette is then ready for instrument calibration or for ATP assay. By employing these special duPont reagents, one can avoid the usual nuisance and delay required for the luciferin-luciferase reagent to "season" in an ice-bath (i.e., to permit dieaway of unwanted background emissions). However, the specially purified luciferin-luciferase powder is shipped in dry ice and must be kept frozen until ready for use. If this is not done, slow decomposition will occur, and the analyst will then have to season his reagent solution just as if it had been prepared from crude firefly-lantern extract.

After the Biometer has been calibrated, samples can be analyzed repetitively at a rate of one analysis each 5 to 10

minutes. If the instrument has been calibrated over a representative and relatively unvarying concentration range, the instrument's five-decade automatic ranging capability usually eliminates the need for even an occasional supplementary sample dilution.

Any extraction solvent or procedure that will rupture cell walls and release the intracellular ATP can be used; e.g., dimethylsulfoxide, perchloric acid, ethanol, butanol, boiling aqueous buffers, freezing and thawing, ultrasonic energy, or abrupt pressure changes. Butanol extraction, however, is recommended by duPont for general use with their Biometer.

Depending on the sample source and on what fraction of the sample is of interest, some combination of selective filtration, centrifugation, or sample washing may be essential before butanol extraction to eliminate unwanted interferences. Also, if butanol or certain other "non-aqueous" cell-rupturing techniques are used, an aqueous phase must be introduced to extract or partition the hydrophilic ATP into an analytically optimum solvent system before injection of the ATP into the luciferin-luciferase reagent.

Although the Biometer is a well constructed and versatile instrument, it also is the highest priced off-the-shelf ATP analyzer presently available. The basic analyzer system (duPont's Catalog No. 760000) costs $4,860. However, this includes 500 disposable reaction cuvettes, 100 pipettor tips, a reagent kit, an ATP kit, and an accessory kit; i.e., sufficient reagents and cuvettes for 500 ATP assays. The accessory kit, in turn, includes an automatic pipettor which uses disposable "measuring chamber" tips (probably some type of Eppendorf pipet), two syringes for sample injection-mixing, 12 septa (syringe-penetrable light seals for the analyzer's cuvette compartment), tools, and spare fuses.

Spare reagent kits, containing reagents and cuvettes for 500 assays (duPont Catalog No. 760145), are also relatively expensive: $170/kit, or 34¢/assay. If a user wishes to employ duPont's Disposable Filter Assemblies (Catalog No. 760312), these cost $125/set of 250; this increases cost/assay by another 50¢ unless a user can safely assume that several successive samples can be handled by each disposable filter before replacing it. Also available for specialized applications are a Bacteriological Kit (Catalog No. 760146; $135) and a Modified Millipore Sterifil™ Filtration System (Catalog No. 760043-902; $75).

According to a duPont Biometer application bulletin (Biometer Flashes, No. 760BF4; August, 1972), it is now

possible to make rapid 1-hour BOD measurements using ATP data plus 1-hour oxygen-uptake-rate data obtained with a D.O. probe. Another issue of Biometer Flashes (No. 760BF1; September, 1970) describes specific applications, including case histories, of ATP measurements in the areas of water ecology and wastewater treatment.

AMINCO's (i.e., American Instrument Company's) CHEM-GLO Photometer - None of AMINCO's bulletins furnish either the weight or dimensions of their CHEM-GLO Photometer (referred to hereafter as the CGP). However, several photographs, depicting this instrument with a recorder, a laboratory technician, etc., permit a reliable estimate of the instrument's size. The CGP appears to be approximately 9 in. wide x 12 in. high x 8 in. deep (23 x 30.5 x 20 cm., respectively), and it probably weighs about 15 to 25 lb (7 to 11 kg). AMINCO also does not mention the CGP's power requirements, but these are almost sure to be negligible (i.e., probably less than 1 amp. at about 115 V and 60 Hz). On the CGP's rear panel are a recorder outlet (set for 50 mv, but adjustable from 5 mv to 60 mv) and an outlet for a 0.6-V oscilloscope. The price of the basic analyzer is $800 (AMINCO Catalog No. 4-7441). However, a repeating dispenser (Catalog No. 4-7444; $300) is recommended for the analysis of multiple samples on a routine basis. This automatic dispenser mounts directly atop the reaction chamber above the CGP's injection port, and it discharges a 0.1-ml increment of firefly reagent from a 5-ml syringe directly into each sample cuvette. Fifty samples can be run before the syringe needs refilling with fresh reagent.

Although the CGP is much less expensive than either of the other two ATP analyzers, it is also not as practical for wastewater-treatment-plant monitoring and control applications. For example:

1. The CGP does not come equipped with a recorder, much less a digital readout. Instead, it possesses only a meter readout similar to those found on low-cost pH meters and filter photometers.

2. The CGP possesses no integrating circuitry. Hence, even if the unit were to be supplemented with an auxiliary stripchart recorder, integration of the recorded dieaway luminescence (i.e., of the area under the stripchart tracing) would necessarily be a manual operation. If the user were to fit the CGP with a recorder for simultaneously measuring and logging merely the initial or peak intensity, then at some later date the height of each peak could only be obtained by careful measurement of

the corresponding recorder tracing (unless the operator had transcribed each CGP meter reading alongside its corresponding stripchart peak at the time each sample had been analyzed).

3. The CGP possesses no automatic ranging, multi-decade, signal-attenuating circuitry. Thus, the operator must estimate each sample's approximate ATP concentration before analysis, then manually adjust the CGP's range selector (i.e., signal attenuator) so that signal output is neither too weak nor too strong. If the operator's estimate is incorrect, the resulting analytical data may be either worthless or of limited reliability.

4. Reagents for ATP analysis are not listed by AMINCO as being furnished with the CGP at time of purchase. In fact, AMINCO does not list these reagents even as extra-cost accessories. Therefore, the user of the CGP is dependent on several auxiliary reagent suppliers and must find out by trial and error which supplier furnishes acceptable reagents for a reasonable cost.

For those users who possess the time, money, and skill to combine and use the CGP with auxiliary components, the CGP's low cost, quality construction, and high sensitivity (i.e., a sensitivity equivalent to that of the Biometer) make it an attractive purchase for specialized applications. For example, AMINCO's Automated System Adaptor (Catalog No. 1501-605; $110) plus their Recorder Adaptor (Catalog No. 4-8266; $295) permits the CGP to be incorporated with the Technicon AutoAnalyzer. Unfortunately, the resulting complex analytical system is expensive, and its operation would require someone skilled in both AutoAnalyzer and bioluminescence techniques. In spite of such difficulties, the following individuals have constructed and successfully operated composite automated instrumentation systems embodying the CGP:

1. Dr. Ivan Stern and Dr. Roger S. Izzo
   Pharmaceutical R&D Division
   Travenol Laboratories
   Morton Grove, Illinois 60053

   Phone: (312) 965-4700, ext. 3953

2. Mr. Ralston W. Reid
   Laboratory Services
   V. A. Hospital
   Hines, Illinois 60141

According to AMINCO, Dr. Grace L. Picciolo of the Goddard Space Flight Center, Greenbelt, Maryland 20771 (Phone: (301) 982-5583), has also automated the firefly reaction with equipment that is even more sophisticated than the AutoAnalyzer. However, since much of Dr. Picciolo's equipment has been custom-built for NASA applications, its cost and complexity would probably exceed the resources of even a large and well-financed domestic STP.

During 1971, AMINCO offered an accessory Integrator-Timer ($1200) for use with the CGP. This combination essentially duplicated the convenient, but manual, analytical system described by J. F. St. John (9) in her detailed study of ATP measurements using the luciferin-luciferase enzyme system. Unfortunately, AMINCO ceased furnishing their Integrator-Timer before it could be put into full-scale production. Indications are, however, that AMINCO's Integrator-Timer will again soon be available in order to make the CGP competitive with the analogous duPont and JRB Inc. ATP analyzers. Because of steadily increasing, nationwide, electronic component and labor costs, AMINCO's new Integrator-Timer is estimated to sell for at least $1500-$1600.

JRB Incorporated's ATP Photometer - This analyzer also is a small, highly sensitive, single-beam photometer (10 x 12½ x 16½ in.; 25 x 32 x 42 cm) and weighs only 30 lb (13.6 kg). Since its intended application is measurement of ATP via the firefly reaction, it is designed for optimum sensitivity to radiation wavelengths of 560 to 580 nm. Instrument power requirement is only 0.3 amp. at 105-130 V and 60 Hz, with an optional choice of 0.15 amp. at 220 V and 50 Hz; no other supporting utilities are needed.

With the use of relatively cheap, nonrefined enzyme, JRB's ATP Photometer can measure as little as 10^{-10} mg of ATP; however, its most useful operating range is 10^{-9} mg ATP/ml to 1 mg ATP/ml. Sample volume can be quite flexible, ranging from 10 µl to 10 ml. After the analyzer has been calibrated, repetitive analyses usually require about 10 minutes/sample.

Unlike duPont's Biometer that measures only peak intensity of emitted radiation, the JRB ATP Photometer is a luminescence-integrating instrument that sums the total quantity of "dieaway" emission over a preselected time interval (either 6 seconds or 60 seconds). According to St. John (loc. cit.), this approach possesses certain inherent advantages:

1. The relationship between ATP concentration and integrated light emission is linear over at least 3 orders of magnitude, with an error of less than 2% relative standard deviation.

2. The sensitivity resulting from integration of low-level dieaway emission is comparable to that afforded by measurement of only the brief, but brilliant, peak-flash emission.

3. Most importantly, at least for STP applications, are the savings in reagent costs that can be realized by using commercially available luciferin-luciferase mixtures without further purification. Typically, the use of such crude reagents is not feasible for peak-intensity ATP analyzers because of the highly variable analytical data that would thereby result. Use of commercial, nonrefined, firefly-lantern extract lowers reagent costs to about 3¢ to 4¢/analysis; whereas, reagents obtained by purification of commercial enzyme extract (or from chemical synthesis of luciferin) cost 30¢ to 35¢/analysis.

The ATP Photometer's lack of the Biometer's automatic ranging, multi-decade, linear response capability could conceivably cause trouble in STP applications if the analyzer were not calibrated at the midpoint of the ATP concentration range of interest. However, this source of trouble is counterbalanced by the Biometer's potential for error stemming from sample injection-mixing, which can only be done through a blind sampling injection port.

Analyzer readout includes a lighted digital display of automatically integrated flash-counts/minute, which is then compared with a standard calibration curve to determine ATP concentration or any related parameter against which the instrument was initially calibrated. Also furnished is a recorder output of 0-100 mv.

Reagent preparation, instrument calibration, ATP extraction, and ATP measurement are basically the same as for the Biometer. Some of the minor differences are as follows:

1. The use of nonrefined firefly-lantern extract.

2. A requirement for standard calibration curves.

3. Cell rupturing and simultaneous ATP extraction by means of boiling "tris" buffer solution (pH 7.7) rather than by butanol, preceded where necessary by filtration of "weak" samples in order to concentrate the cellular material to an analytically meaningful concentration level.

4. Either 6-second or 60-second luminescence-integration, rather than measurement of only peak intensity.

Cost of the basic analyzer is $3,950. This does not include $28 for the basic Reagent Kit which consists of 5 vials of firefly extract, 2 vials of preweighed ATP standard, 3 vials of tris buffer powder, and 100 scintillation vials (i.e., cuvettes). Useful auxiliary equipment includes a Biopette dispensing pipet ($59.95) and 1000 disposable Biopette tips ($39.00). Individual quantities of replacement reagents are as follows:

1. Firefly extract, 10 vials, 50 mg/vial - $20

2. Preweighed ATP standard, 10 vials, 1 mg/vial - $6

3. 100 scintillation vials - $15

4. pH 7.7, tris buffer, 100 g - $12

Additional accessories are also available for mobile and oceanographic applications and for medical or enzyme-kinetic studies.

To any user of ATP-measuring equipment, the importance of the following statement, which appeared in J. B. St. John's previously cited publication, cannot be overestimated: "... before any system based on the luciferin-luciferase reaction can be employed for quantitation of ATP in a biological extract, the effect of the biological extract on the response of a known quantity of ATP must be determined before a standard curve can be used instead of constant addition." Although Mrs. St. John used the term "standard curve", her warning applies just as strongly to the duPont Biometer (which requires no standard curve) as to the JRB ATP Photometer. Although the firefly reaction is rapid and highly sensitive for ATP, it is not absolutely specific for ATP and is influenced by a wide variety of factors. For example, the reaction is inhibited by certain buffers, nucleotide phosphates (mono-, di-, and tri-), inorganic salts, ethanol, high magnesium concentrations at low concentrations of ATP, and even by ATP itself at low magnesium concentrations.

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